A biochemically defined system for coding joint formation in V(D)J recombination.

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Public Summary:
All human DNA repair processes require a nuclease to remove damaged DNA. Metaphorically speaking, a nuclease is like a surgical scalpel that removes the damaged tissue, but in this case the damage is at the molecular level in the DNA. NHEJ is the name of one of the five major DNA repair types. We had previously identified the major nuclease for NHEJ. In this paper, we identify a secondary nuclease for this pathway. The significance of this for stem cell gene correction is as follows: if we could block the NHEJ pathway, this would improve gene targeting by directing the correction process along the pathway (homologous recombination) that achieves the gene correction. By identifying and understanding all of the components of the relevant repair pathways, we have a more intelligent way of improving gene targeting efficiency

Scientific Abstract:
V(D)J recombination is one of the most complex DNA transactions in biology. The RAG complex makes double-stranded breaks adjacent to signal sequences and creates hairpin coding ends. Here, we find that the kinase activity of the Artemis:DNA-PKcs complex can be activated by hairpin DNA ends in cis, thereby allowing the hairpins to be nicked and then to undergo processing and joining by nonhomologous DNA end joining. Based on these insights, we have reconstituted many aspects of the antigen receptor diversification of V(D)J recombination by using 13 highly purified polypeptides, thereby permitting variable domain exon assembly by using this fully defined system in accord with the 12/23 rule for this process. The features of the recombination sites created by this system include all of the features observed in vivo (nucleolytic resection, P nucleotides, and N nucleotide addition), indicating that most, if not all, of the end modification enzymes have been identified.